Haruo OKUNO*: **Electron microscopical study on antarctic diatoms** (1)

奥 野 春 雄*：南氷洋産珪藻の電子顯微鏡的研究 (1)

Introduction

I was forwarded three tubes of antarctic planktons from Mr. Fumitaka Tsubata of the Hakodate Marine Meteorological Observatory. The planktons were collected by himself with a Hensen's quantitative net from the board of the whale-boat "Second Ten'yō-maru' of the Taiyō Fishery Company. Of the planktons, the stations, date, etc. are shown in the following table.

Station		Depth from water surface (m)	Date
Number	Latitude & Longitude		
27	66° 30′ S.; 166° 52′ W.	0-25	Jan. 18, 1950
39	65° 34′ S.; 158° 23′ W.	〃	Jan. 29, 1950
62	67° 39′ S.; 164° 50′ W.	〃	Feb. 27, 1950

The present three tubes include almost diatoms only, and other phytoplanktons and zooplanktons are very rarely found. By means of the electron microscope, I studied in detail the super-fine structures of several kinds of diatom-frustules found in them. In the present research several types of frustule-pores described in the chapter "Description of fine structures" are brought to light. The electron micrographs presented here were partly photographed at the Yasuzumi Laboratory of Osaka University and at the Researching Department of Shimadzu Works, and partly at our own laboratory.

I wish to express my hearty thanks to Mr. Fumitaka Tsubata, who kindly sent me the valuable specimens, and many thanks are also due to Dr. Gompachirō Yasuzumi of Osaka University and Dr. Shin'ichi Shimadzu of Shimadzu Works, who gave me the opportunity of taking many electron micrographs at their laboratories. Further I take this opportunity of thanking to my assistant Mr. Kiichirō Kurosawa for his aid in many

* Botanical Institute, Faculty of Textile Fibers, Kyoto University of Industrial Arts and Textile Fibers, Kamikyo-ku, Kyoto. 京都工藝繊維大學 繊維學部 植物學研究室.

ways. At last I also wish to give public expression of my thanks to my wife, Shigeko Okuno, who has in economical and other ways supported me in the work.

Preparation of diatom-frustules for electron microscopy

Diatoms preserved in 5 per cent formalin solution, were dropped on a slide glass through a pipet and dried gently on an alcohol lamp. Searching under the light microscope, the valve or the fragment of the valve, which seems to be suitable for the electron microscopy, is caught with the help of a sharp needle, and transferred on the collodion membrane of the sample holder. Then the collodion membrane on which the diatom is resting, was exposed two or three seconds to aqueous vapour to fix the diatom on it. When a large frustule was prepared, it was fixed on the holder without a holding membrane in such a manner that one part of the frustule is projected over the pore of the holder to be exposed to the electron beam. I call such a preparation without a holding membrane a "direct preparation" in comparison with the collodion preparation. The direct preparation too, was exposed to aqueous vapour for fixing the diatom on the holder. As the complete frustules, which have both upper aud lower valves, are generally not penetrable to the electron beam, I always prepared an isolated single valve or a fragment of a valve for the electron microscopy. When such a suitable fragment which seems to be penetrable to the electron beam cannot be found on the slide, the complete frustule was carefully seperated into single valves, or broken into fragments of a single valve with a sharp neelde under the light microscope. Such a micro-operation as catching or breaking diatom-frustules under the light microscope, required me considerable skill and perseverance. For separating protoplasm from the cells, no special treatment was applied. In present experiments, the fragments of diatom-frustules obtained from the cells burnt on an alcohol lamp, were in many cases sufficiently penetrable to the electron beam.

Description of fine structures

Asteromphalus heptactis (Bréb.) Ralfs (Pl. I, figs. 1-1") Hustedt, Kieselalg. I: 494, figs. 175, 177 (1930); Mills, Index Diat.: 218 (1933).

L. M. S.* (figs 1, 1') Valves subcircular, about 30-90 μ in diameter. The cen-

* L. M. S.: Light microscopic structures.

tral hyaline area is somewhat excentric and in diameter about one fourth to one third of the valve. From the centre radiate 6-7 ribs reaching the inner margins of the segments. Areolas 6-7 in 10 μ, arranged in three directions.

E. M. S.** (fig. 1") A fragment of a valve was prepared as a collodion preparation. Fine structures: The areolas in the segments are locular. A loculus is hexagonal, with a thin outer sieve membrane and a half closed inner cover membrane. A sieve membrane is very thin, penetrable to the electron beam, perforated by several minute sieve pores. Sieve pores roundish, about 200 mμ in diameter, occur 3-4 in 1 μ. On the electron image, the sieve pores at the marginal part of a loculus cannot be seen, concealed by the cover membrane. The central pore of the cover membrane is roundish, about 500-900 mμ in diameter. The interspaces between segments are somewhat penetrable to the electron beam, but not porous. The light micrograph in Fig. 1', and the electron micrograph in Fig. 1", were obtained from the same valve. Namely, after photographed in the electron microscope, the valve was transferred with the help of a sharp needle on a cover glass and was mounted with colophonium on a slide glass to be photographed under the light microscope.

St. no. 27 (+++); no. 39 (+); no 62 (+++).

Coscinodiscus lunae Ehrenberg ? (Pl. I, figs. 2-2") Mills, Index Diat.: 484 (1933). *Coscinodiscus cycloteras* Castracane, in Rep. Voy. Challenger, Bot. 2: 161, pl. 22, fig. 8 (1886).

L. M. S. (figs. 2, 2') valves circular, about 22-47 μ in diameter. Marginal zone striated, striae about 18-20 in 10 μ. Within the marginal zone, a belt of small granules about 10 in 10 μ, runs around the valve. Pores about 7-9 in 10 μ, almost equal in diameter, arranged in longer and shorter radiating rows. The density of pores are very variable in different cells. The central area absent.

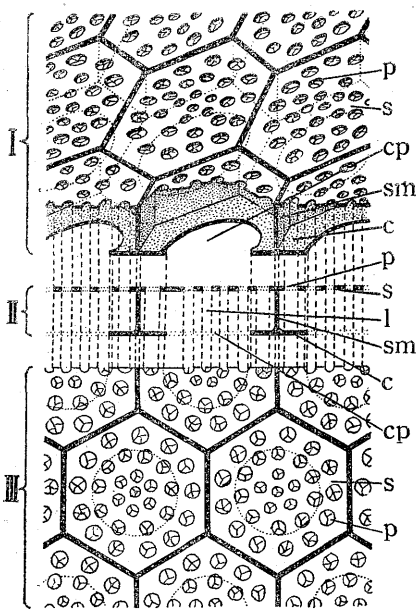
Remarks: Our specimens differ from those described and sketched by Castracane in the following points. In our specimens, the marginal zone striated, which in Castracane's sketch not striated. The belt of granules on the submarginal zone, in our specimens consists of one ring, while Castracane's sketch shows double rings.

E. M, S. (fig. 2") An isolated single valve was prepared as a collodion

** E. M. S.: Electron microscopic structures.

preparation. Fine structures: The central part of the valve was observed in the electron microscope. The pores are roundish, about 250 mμ in diameter, and very simple in their structures in comparison with those of other *Coscinodiscus*. Such simple pores as in this species are rather exceptional in *Coscinodiscus*. The sieve membranes seem to be homogene, not porous. The light micrograph in Fig. 2' and the electron micrograph in Fig. 2", were obtained from the same valve.

St. no. 27 (+); no. 39 (++); no. 62 (+).



Text fig. 1. Diagram of loculi of *Coscinodiscus oculus-iridis*. I, Viewed obliquely from above, partly with the cross-section. II, Cross-section. III, Viewed vertically from above. c, Cover membrane: cp, Cover pore. l, Loculus. p, Sieve pore. s, Sieve membrane. sm, Side membrane.

Coscinodiscus oculus-iridis Ehrenberg (Pl. I, figs. 3, 3'; Text fig. 1) Hustedt, Kieselalg. **1**: 454, fig. 252 (1930); Mills, Index Diat. 491 (1933).

L. M. S. (fig. 3) The valve circular, about 180 μ in diameter. The central rosette consist of several large, polygonal areolas. In the other parts of the valve, areolas 3-3.5 in 10μ arranged in radiating rows. Areolas decreasing to 2.5-3 at the submarginal part, and again at the margin increasing to 4 in 10 μ. Marginal zone narrow, striated, striae about 6 in 10 μ.

E. M. S. (fig. 3'; Text fig. 1) An isolated single valve was prepared as a direct preparation. Fine structures: The valve has hexagonal loculi. Sieve membranes of the loculi have concentrically arranged sieve pores which increase their diameters from 100 to 350 mμ in approaching the border of the oculi. A sieve pore is divided by minute septae into 2-4 micropores. A cover membrane has a round central opening about 2-2.3 μ in diameter. The structures of the loculi are shown diagrammatically in Text fig. 1. The light micrograph in fig. 3, and the electron

micrograph in fig. 3', were obtained from the same valve.

St. no. 27 (+).

Coscinodiscus sp. (Pl. I, figs. 4, 4')

L. M. S. (fig. 4) Valves circular, almost flat, about 40 μ in diameter. Areolas about 8 in 10 μ, gradually decreasing their diameters to the margin of the valve. In the centre of the valve, areolas absent. The rows of areolas are bundled in radial groups. Marginal zone indistinct. The present doubtful form is very similar to *Coscinodiscus Rothii* var. *subsalsa* (Hustedt, Kieselalg. **1**: 402, fig. 212) and *Cosc. curvatulus* var. *minor* (Hustedt, l.c. 409, fig. 217), but it differs from the former by the indistinction of the marginal zone, and from the latter by the presence of parallel shorter radiating rows between the longer rows.

E. M. S. (fig. 4') An isolated single valve was prepared as a collodion preparation. Fine structures: The valve is locular. The sieve membrane is thin, with round sieve pores about 8 in 1 μ. The diameter of a sieve pore is about 75 mμ. On a sieve membrane, the sieve pores are arranged somewhat concentrically. But the directions of these lines of pores are not common to the different loculi. The cover membrane is thick, with a roundish opening in the centre. The diameters of the openings are larger (about 600 mμ) in the centre of the valve, and smaller (about 300 mμ) at the margin. The light micrograph in fig. 4 and the electron micrograph in fig. 4', were obtained from the same valve.

St. no. 39 (+).

Guinardia blavyana Peragallo (Pl. I, figs. 5, 5') Hustedt, Kieselalg. **1**: 564, fig. 323 (1930); Mills, Index Diat.: 823 (1934).

L. M. S.(fig. 5) Frustules cylindrical about 40–55 μ in diameter. The annuli narrow, about 3 μ high. The ends of annuli wedge shaped, alternately inserting with the opposite ones. The longitudinal bars in an annulus about 14 in 10 μ.

E. M. S. (fig. 5') A part of the girdle was prepared as a direct preparation. Fine structures: The annuli are membranaceous, with longitudinal thickenings or bars. The bars, variable in breadth, are thinned down to the sieve membranes. Sieve membranes are very thin, scattered with minute dots, about 50 mμ in diameter. These minute dots are supposed to be the primitive sieve pores with thin membranes.

St. no. 62 (+++).

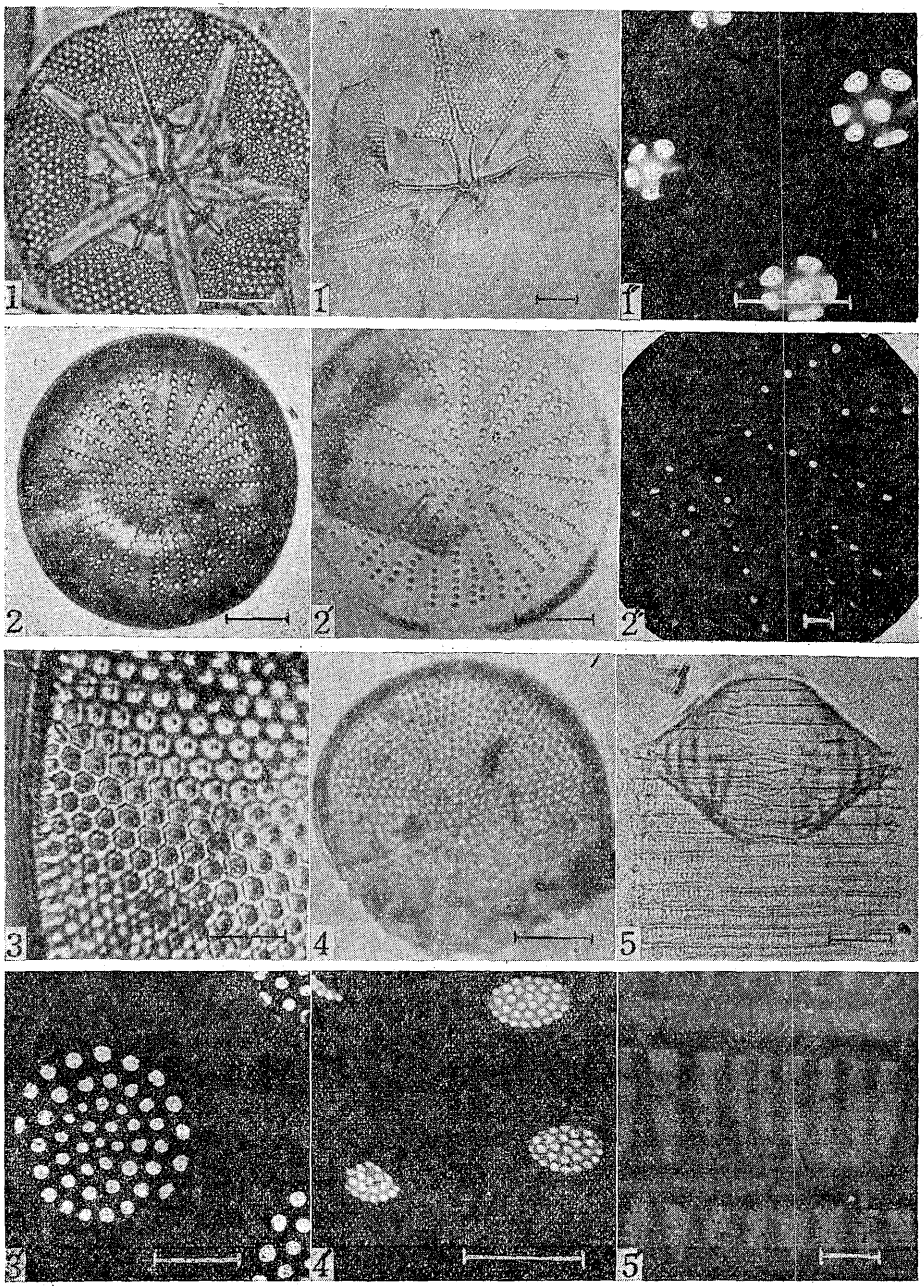


Plate I. Figs. 1, 1' 2, 2', 3, 4, 5……Light micrographs. Scales : 10 μ.
1'', 2'', 3', 4', 5' ……Electron micrographs. Scales : 1 μ.